Stem Cell Research Opportunities in the Musculoskeletal System

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Introduction

Musculoskeletal MR imaging is widely used in everyday clinical practice to investigate disorders of soft tissue masses, bones, and joints (1-3). The emergence of stem cell therapy is creating new opportunities for treatment of musculoskeletal disorders with MR intervention and follow-up. Two lines of cell therapy can be explored. The first line is using stem cells that normally give rise to cells of the musculoskeletal system, and that can thus replace malfunctioning cells and form new tissue at the site of injury. The most well-studied stem cell in the musculoskeletal arena is the mesenchymal stem cell (MSC) (also called bone marrow stromal cell, to discriminate it from hematopoietic bone marrow cells). These MSCs have an amazingly wide capability of cell differentiation, and are the cells that form cartilage, bone, muscle, support tissue, tendons, and (redundant?) adipose tissue (see Figure 1).

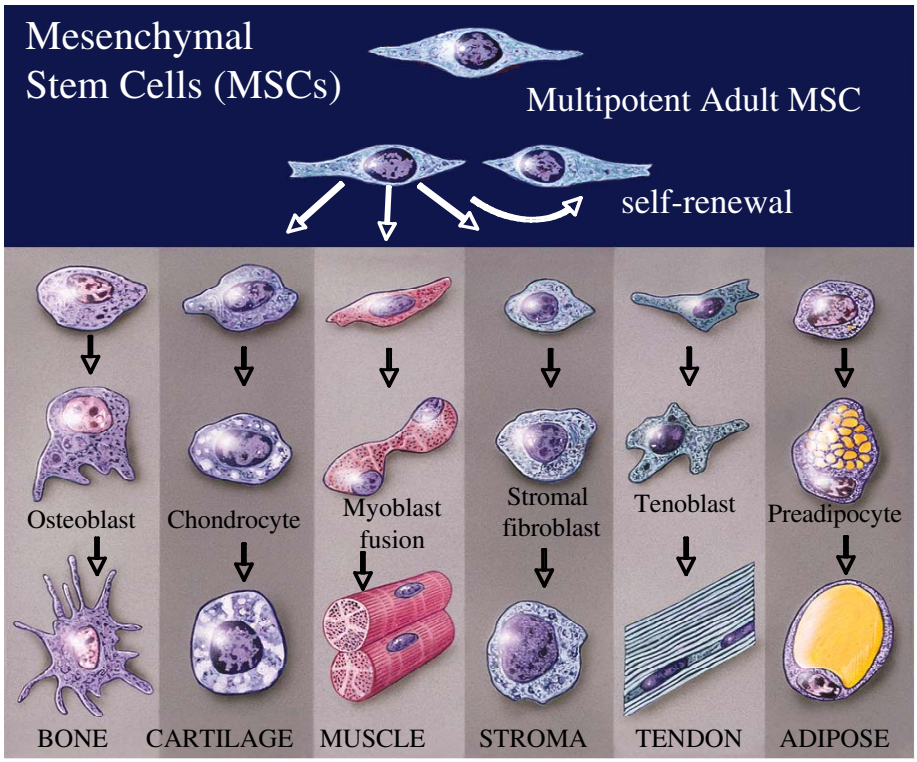

Figure 1: Multipotency of adult MSCs. A key feature of stem cells, including MSCs, is that in parallel with cell differentiation they self-renew, so that an exact copy of an undifferentiated cell is being produced and retained throughout life. Figure courtesy of Osiris Therapeutics, Inc.

The second line of cell therapy that is being explored is to boost the patient's own immune system to fight cancer, applicable to musculoskeletal tumors, although so far most immune stimulation/cancer vaccination studies have been conducted in patients with melanoma and prostate cancer. The cells of choice can be tumor antigen-specific T lymphocytes, activated killer cells, or dendritic cells (4).

Under the right circumstances, MSCs may "home" to injuries, engraft and differentiate into various cell types (5). Clinical trials using MSCs for musculoskeletal repair, including bone regeneration (6,7) and joint repair (8) have now being started. A few specific potential applications and examples will now be further discussed in detail below.

Stem cell opportunities in bone repair

A striking example of the therapeutic use of autologous adult MSCs, that may sound like science fiction, was published in 2004 (9). A cancer patient had a large center part of his jaw removed 9 years prior. A titanium cage was molded that matched the missing piece, and was seeded with MSCs along with bone morphogenic protein. This engineered scaffold was then implanted in the patient's back, and monitored for bone formation using a bone-seeking radiotracer and scintigraphy. Following sufficient growth of new bone, the mandibular scaffold was excised and implanted between the two pieces of jaw. By the 4^{th} week post-transplantation, the patient could enjoy his first dinner in 9 years (reportedly a Bratwurst sandwich, with the study having been performed in Germany). Even with his edentulous jaws, mastication was now possible, and the stem cell approach prevented the occurrence of a secondary bone defect.

Stem cell opportunities in arthritis

Rheumatoid arthritis (RA) and osteoarthritis (OA) remain incurable and difficult to treat. In these cases, the articular cartilage frequently incurs damage because of injury or disease, but has very limited powers of regeneration. However, injury that penetrates the cartilage layer and subchondral bone, causing rupture of the vasculature and marrow, will allow the influx of MSCs into the lesion. These multipotent cells locally differentiate and synthesize fibrocartilage repair tissue. Although this tissue will offer temporary symptomatic relief, with time and use, it generally fails. Several proteinaceous factors have activities that may stimulate the differentiation of MSCs toward the synthesis of an improved repair tissue, but they are difficult to apply effectively. Cultures of MSCs genetically modified to constitutively express certain growth factors, such as TGF-β1 and BMP-2, will undergo chondrogenesis in aggregate pellet cultures (10-12). From these findings, arise the overall hypotheses that gene transfer can be used as a means to achieve persistent synthesis of specific proteins within a cartilaginous lesion, and that delivery of certain stimulatory molecules in this manner can be used to augment the differentiation of MSCs toward chondrogenesis *in vivo*.

Stem cell opportunities in muscular dystrophy

Muscular dystrophy is an inherited disease that is known to result in skeletal muscle weakness and cardiac and respiratory failure, resulting from chronic bouts of muscle damage and regeneration; eventually exhausting the endogenous pool of stem cells leading to organ failure and death. A successful treatment for such muscular diseases will need to meet several criteria: 1) prevent cell necrosis, 2) increase muscle mass and force, and 3) improve the structural integrity of the remaining cells. Stem cell transplantation has the potential to meet these criteria, since stem cells can serve as vehicles to deliver therapeutic or missing genes in addition to

increasing the myogenic capacity of the tissue. Moreover, in some cases stem cell transplants have been shown to play an important therapeutic role independent of cell replacement or transdifferention/fusion, by providing essential nutrients and mechanical support to the damaged tissue (13).

Initial transplantation strategies in muscular dystrophy focused primarily on the delivery of myoblasts to the dystrophic muscle. Since its identification, the satellite cell or myoblast has been considered an adult skeletal muscle stem cell. Initial myoblast transfer studies showed great promise and demonstrated the ability of myoblast cells to increase muscle mass and restore function following muscle necrosis and damage (14-16). Unfortunately the therapeutic efficacy of early myoblast transfer studies was limited by massive myoblast cell death observed immediately following *in vivo* delivery (17). Recent interest has focused on identifying novel stem cell populations that escape this early period of cell death and may be better suited for transplantation therapies.

Muscle-derived stem cells (MDSC) have shown a potential ability to repair dystrophic skeletal muscle (18-20). This cell population can undergo *in vivo* differentiation to regenerate lost myofibers and restore dystrophin expression (18,21-25).They are also capable of reconstituting the hematopoietic stem-cell compartment of lethally irradiated dystrophic mice (26) and forming bone (27). These combined characteristics are indicative of a unique stem cell population with a less committed phenotype than the traditional primary myoblasts used in early transplant studies. Intramuscular injection of normal muscle-derived stem cells into the murine model of Duchenne muscular dystrophy (*mdx* mice) produces a 10-fold increase in dystrophin positive myofibers compared to the same procedure done with normal myoblasts (22,24). Moreover, dystrophin expression persists up to 90 days and results in histopathological correction, as demonstrated by a decrease in the number of central nucleated fibers. These early results indicate that MDSC could provide a source of cells for therapeutic transplants in dystrophic muscle. In addition, MDSC cells have immune-privilege properties permitting them to avoid immune rejection (24). Finally, MDSC also have properties that permit systemic delivery through the circulatory system (28). Arterial delivery strategies have been explored to provide more global delivery of the cell grafts to dystrophic muscle. MDSCs will migrate from the vasculature to engraft in dystrophic muscle (19,22,26,28). Recent data indicate that this homing pathway involves the interaction of vascular endothelial cells and L-selectin expressed by the migrating stem-cell population (28) and damaged muscle cells (29-31).

Additional adult muscle progenitor cells have also been isolated from non-muscle tissue. Whole bone marrow, hematopoietic cells, adipocytes, MSCs (above), and fibroblasts have all been shown to have the capacity to form muscle under the right conditions. In 1998 it was first reported that bone-marrow progenitor cells, including adherent and nonadherent populations, are capable of participating in skeletal muscle repair in normal mice after cardiotoxin-induced damage (32). It was also shown that bone-marrow cells, including purified hematopoietic progenitor cells, can contribute to the regeneration of skeletal and cardiac muscle in *mdx* mice, in which these striated muscles undergo continual remodeling (33,34). Gussoni et al reported that this process can also be observed in human muscle (35).

Finally, embryonic and fetal stem cells also have shown the potential to rescue dystrophic muscle. Intra-arterial injection of wild-type mesoangioblasts (vessel associated fetal stem cells) in a murine model of limb girdle muscle dystrophy resulted in expression of the missing sarcoglycan in more than 50% of soleus muscle fibers. In addition, the mesoangioblasts restored sarcolemmal integrity and resulted in functional recovery (29). This is especially important for

the treatment of essential muscles such as the diaphragm, impairment of which results in severe respiratory problems in muscular dystrophy (36).

MR imaging opportunities

The design and improvement of the stem cell-based therapies will be greatly facilitated by the development of sensitive, non-invasive, and non-destructive techniques for tracking stem cells following implantation or infusion. It is here where the opportunity exists to use cellular MR imaging in translational and clinical stem cell research. Using superparamagnetic iron oxides (SPIO) and in particular the clinical formulation Feridex® it has now been reasonably well established that cell therapy, including the delivery and migration of labeled cells, can be reliably monitored by MR imaging (37). That is, when there is no significant cell death and subsequent label uptake by macrophages, and when cells are not diluting out the label by cell division within the timeframe of imaging.

SPIO contrast agents have been utilized to monitor therapeutic muscle stem-cell transplants in rodents (38,39) and in a murine model of Duchenne/Becker's muscular dystrophy (*mdx* mice) (40). These studies were performed with a subclone of MDSCs, mc13, that has the capacity to efficiently regenerate skeletal muscle in *mdx* mice following a single intramuscular injection (22). Mc13 cells are engineered to express the mini-dystrophin gene and the β-galactosidase (LacZ) reporter gene, which allows for correlative histological studies. SPIO Labeled mc13 cells were transplanted into the gastrocnemius-plantaris-soleus muscle group of 6 weeks old *mdx* mice. High-resolution MRIs were obtained 24 hrs, 2, 4, and 11 days post-injection. Distinct regions of signal hypo-intensity were identified in the posterior musculature of animals receiving labeled cell transplants at all time points (Fig 2; (40)). Control animals receiving unlabeled cell transplants displayed homogenous images, without the regions of hypo-intensity seen in the experimental animals. Engrafted cells were detected by analysis of β-galactosidase activity, dystrophin expression, and iron content. LacZ expressing fibers were readily identified in regions corresponding to the hypo-intense regions in MR images. Additionally, Prussian blue staining of consecutive serial sections revealed the presence of iron accumulation in many of the LacZ positive fibers, confirming the correlation between the histological location of the cells and MR images. Immunostaining for mini-dystrophin indicated that the engrafted cells restored membrane dystrophin expression and were therefore potentially therapeutic (40).

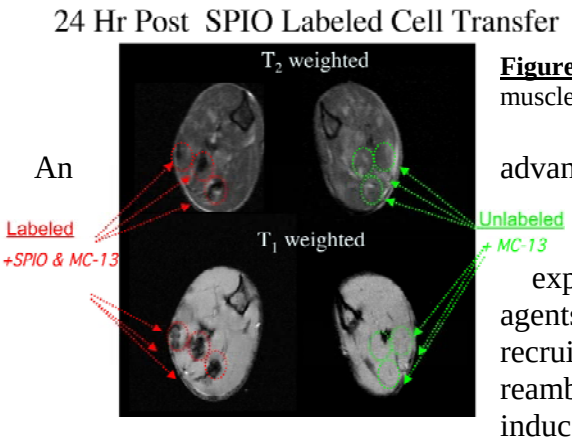

Figure 2: High Resolution T_1 and T_2 contrast generated in dystrophic muscle following SPIO labeled MC13 transfer.

An advantage of cell therapy is the belief that during tissue damage stem cells will home to regions of tissue regeneration. Muscle damage results in the expression of a cascade of myogenic and chemotactic agents which are essential for muscle regeneration and the recruitment of stem cells (41). As shown above reambulation/reloading following cast immobilization, induces massive muscle damage in the mechanically loaded soleus muscle (42). The rapid regeneration of muscle following this type of damage indicates that the coordinated expression of endogenous factors necessary for myogenic cell

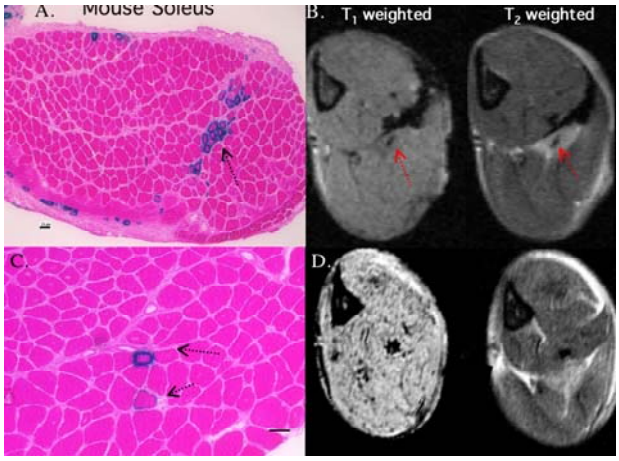

Figure 3: Targeting of muscle derived stem cells to the mouse soleus following casting and reambulation. Note the hyperintense regions due to muscle damage on T_2 weighted images, and the hypointense regions corresponding to cell deposition on both T_1 and T_2 weighted images.

migration, proliferation, and differentiation are present in this model. In order to track cell delivery, SPIO labeled stem cells were administered by direct intramuscular injection and were observed by MRI as regions of hypo-intensity in the regenerating soleus as well as other posterior hindlimb muscles (Fig. 3). Engrafted cells were confirmed by LacZ activity as well as iron content. LacZ expressing fibers were identified in regions corresponding to the hypo-intense regions on T_2-MRI (Fig 3A). Additionally, Prussian blue staining of corresponding serial sections revealed the presence of iron accumulation in LacZ positive fibers. Mc13 cell engraftment was also monitored in this model following arterial delivery. Consistent with previous findings of vascular delivery of SPIO labeled cells (38), small, punctuate areas of decreased signal intensity were seen only in the limb musculature of the leg that received labeled cell infusion (Fig 3D). The contra-lateral limb and control limbs injected with unlabeled cells did not demonstrate this characteristic pattern. Histological analyses of the leg musculature showed stem cells within the vasculature, distributed in patterns corresponding to the MR images. X-gal staining confirmed the presence of stem cell integration in the soleus following vascular delivery (Fig 3C).

One potential pitfall is the effect of iron loading upon cell function. We have shown that SPIO labeling is not toxic to muscle derived stem cells and does not alter the normal growth rate (38,40). The labeled cells differentiated to mature, multinucleated myotubes at rates comparable to unlabeled cells (38). The resulting myotubes displayed intracellular iron accumulation throughout the length of the myotubes and were otherwise morphologically indistinguishable from unlabeled myotubes. Immunofluorescent analysis of alpha-actinin and desmin expression also revealed that labeled myotubes contain normal sarcomeres (43). On transmission electron microscopy images, electron dense areas indicative of iron accumulation could be seen in the endosomal compartments (38). As previously suggested, trapping of iron-oxide inside the endosome reduces the chance of Fenton-like reactions in the myoplasm and the containment of the iron until it can be metabolized (44). In agreement with this hypothesis, ferumoxide accumulation did not affect cellular viability or alter the normal growth rate of labeled cells *in vitro*. However, while overall Feridex-labeling does not appear to affect cell viability, proliferation, and differentiation, in the case of chondrocytic differentiation of MSCs a marked inhibition of proteoglycan production (a hallmark of chondrogenesis) has been observed (45). As this was found to be dependent on the labeling dose (lower iron loads did not impair chondrogenesis) (46), careful titration of label and pre-in vivo assessment is warranted for specific cell applications.

Ultimately, it is the fate of the viable transplanted stem cells which will determine the efficacy of the treatment such that noinvasive methods of monitoring gene transfer need not only be capable of monitoring the initial delivery of cells but also if tissue integration/regeneration

occurs. Whereas cell labeling will generate that highest initial contrast, a limiting factor of this strategy is the ultimate fate of the label. In order to track cell migration and integration, stem cells will be engineered to express an MR probe under a tissue specific or conditional promoter. A number of approaches have been developed to monitor gene expression *in vivo* using MRI and spectroscopy (22,46-49). MR strategies have included activated contrast agents (50), the targeting and expression of cell surface receptors (51), antibodies labeled with contrast agents (52), and the expression of unique genes (53-56). All these approaches aim to present a unique signature in the target tissue either by generating MR contrast or by the expression of an absent or foreign metabolite. Unique marker genes have been utilized to monitor gene expression in tumor xenographs using cytosine deaminase from yeast grown in animals (57) and viral delivery of genes encoding iron loading proteins in the brain (56). The expression of mammalian genes in tissue that normally does not contain creatine kinase (CK) has also been used to detect gene expression *in vivo*. Both transgenic (53) and viral delivery methods (54) have demonstrated that the expression of CK in murine livers can result in the production of phosphocreatine and is detectable *in vivo* using ^{31}P-MR spectroscopy. Similarly, we have previously developed a marker gene that results in a novel ^{31}P metabolite (phosphoarginine) in CK containing tissues, i.e. skeletal and cardiac muscle. One of the latest developments is the construction of an artificial lysine-rich protein (LRP), chock full of amide protons, that can be detected by chemical exchange saturation transfer (CEST) imaging (58). As the contrast relies on direct detection of the exchangable amide protons, this is a prototype example of an endogenous reporter that does not need administration of a substrate or contrast agent, and can be swtiched “on” and “off” at will by applying an off-radiation pulse.

The unique strength of MR imaging is that it can not only be applied for visualizing stem cell injection and cell migration, but also for follow-op of regenerating tissue induced by stem cells, e.g. meniscal tear repair (59). If succesful, there are plenty of opportunities for reimbursement of this specific application, given the existence of both expensive race horses and soccer players. Along these lines, ^{1}H single and double Quantum MRI has been used to evaluate tendon regeneration following implantation of collagen sponges seeded with adult MSCs (60). As for the future of clinical stem cell therapy and MR imaging, an important development has been the rise of MR fluoroscopy using MR-compatible catheters (61) and the use of “open” MR scanners, allowing the interventional radiologist to perform MR-guided stem cell injections in real time. As both the MR-labeled stem cells and the injection target (i.e. tumor mass or specific joint/tendons) can be visualized, one can assure verification of accute delivery and re-inject cells if needed. Although many invasive needle procedures are being performed by radiologists (i.e., arthroscopic joint injections, percutaneous tumor biopsies), the difficulty with targeted injections should not be underestimated, as a recent clinical cellular MR tracking study has shown that even experienced radiologists can miss their target organ in half the patients when performed solely under ultrasound guidance (62). In summary, with the tools being in place, there are plenty of research opportunities in the musculoskeletal system.

References

1. Ma LD, Frassica FJ, Bluemke DA, Fishman EK. CT and MRI evaluation of musculoskeletal infection. Crit Rev Diagn Imaging 1997;38(6):535-568.
2. Ma LD. Magnetic resonance imaging of musculoskeletal tumors: skeletal and soft tissue masses. Curr Probl Diagn Radiol 1999;28(2):29-62.

3. Mosher TJ, Dardzinski BJ, Smith MB. Human articular cartilage: influence of aging and early symptomatic degeneration on the spatial variation of T2--preliminary findings at 3 T. Radiology 2000;214(1):259-266.
4. Figdor CG, de Vries IJ, Lesterhuis WJ, Melief CJ. Dendritic cell immunotherapy: mapping the way. Nat Med 2004;10(5):475-480.
5. Prockop DJ, Gregory CA, Spees JL. One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. Proc Natl Acad Sci U S A 2003;100 Suppl 1:11917-11923.
6. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci U S A 2002;99(13):8932-8937.
7. Alhadlaq A, Elisseeff JH, Hong L, Williams CG, Caplan AI, Sharma B, Kopher RA, Tomkoria S, Lennon DP, Lopez A, Mao JJ. Adult stem cell driven genesis of human-shaped articular condyle. Annals of Biomedical Engineering 2004;32:911-923.
8. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 2003;48(12):3464-3474.
9. Warnke PH, Springer IN, Wiltfang J, Acil Y, Eufinger H, Wehmoller M, Russo PA, Bolte H, Sherry E, Behrens E, Terheyden H. Growth and transplantation of a custom vascularised bone graft in a man. Lancet 2004;364(9436):766-770.
10. Palmer GD, Steinert A, Pascher A, Gouze E, Gouze JN, Betz O, Johnstone B, Evans CH, Ghivizzani SC. Gene-induced chondrogenesis of primary mesenchymal stem cells in vitro. Mol Ther 2005;12(2):219-228.
11. Evans CH, Robbins PD, Ghivizzani SC, Wasko MC, Tomaino MM, Kang R, Muzzonigro TA, Vogt M, Elder EM, Whiteside TL, Watkins SC, Herndon JH. Gene transfer to human joints: progress toward a gene therapy of arthritis. Proc Natl Acad Sci U S A 2005;102(24):8698-8703.
12. Caplan AI. Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. Tissue Eng 2005;11(7-8):1198-1211.
13. Goodell MA. Stem-cell "plasticity": befuddled by the muddle. Curr Opin Hematol 2003;10(3):208-213.
14. Wernig A, Zweyer M, Irintchev A. Function of skeletal muscle tissue formed after myoblast transplantation into irradiated mouse muscles. J Physiol 2000;522 Pt 2:333-345.
15. Wernig A, Irintchev A, Lange G. Functional effects of myoblast implantation into histoincompatible mice with or without immunosuppression. J Physiol 1995;484 (Pt 2):493-504.
16. Irintchev A, Langer M, Zweyer M, Theisen R, Wernig A. Functional improvement of damaged adult mouse muscle by implantation of primary myoblasts. J Physiol 1997;500 (Pt 3):775-785.
17. Cossu G, Mavilio F. Myogenic stem cells for the therapy of primary myopathies: wishful thinking or therapeutic perspective? J Clin Invest 2000;105(12):1669-1674.
18. Jankowski RJ, Deasy BM, Huard J. Muscle-derived stem cells. Gene Ther 2002;9(10):642-647.
19. Torrente Y, Tremblay JP, Pisati F, Belicchi M, Rossi B, Sironi M, Fortunato F, El Fahime M, D'Angelo MG, Caron NJ, Constantin G, Paulin D, Scarlato G, Bresolin N. Intraarterial injection of muscle-derived CD34(+)Sca-1(+) stem cells restores dystrophin in mdx mice. J Cell Biol 2001;152(2):335-348.
20. Deasy BM, Huard J. Gene therapy and tissue engineering based on muscle-derived stem cells. Curr Opin Mol Ther 2002;4(4):382-389.
21. Ikezawa M, Cao B, Qu Z, Peng H, Xiao X, Pruchnic R, Kimura S, Miike T, Huard J. Dystrophin delivery in dystrophin-deficient DMDmdx skeletal muscle by isogenic muscle-derived stem cell transplantation. Hum Gene Ther 2003;14(16):1535-1546.
22. Lee JY, Qu-Petersen Z, Cao B, Kimura S, Jankowski R, Cummins J, Usas A, Gates C, Robbins P, Wernig A, Huard J. Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. J Cell Biol 2000;150(5):1085-1100.
23. Huard J, Acsadi G, Jani A, Massie B, Karpati G. Gene transfer into skeletal muscles by isogenic myoblasts. Hum Gene Ther 1994;5(8):949-958.
24. Qu-Petersen Z, Deasy B, Jankowski R, Ikezawa M, Cummins J, Pruchnic R, Mytinger J, Cao B, Gates C, Wernig A, Huard J. Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. J Cell Biol 2002;157(5):851-864.
25. Huard J, Cao B, Qu-Petersen Z. Muscle-derived stem cells: potential for muscle regeneration. Birth Defects Res Part C Embryo Today 2003;69(3):230-237.
26. Cao B, Zheng B, Jankowski RJ, Kimura S, Ikezawa M, Deasy B, Cummins J, Epperly M, Qu-Petersen Z, Huard J. Muscle stem cells differentiate into haematopoietic lineages but retain myogenic potential. Nat Cell Biol 2003;5(7):640-646.

27. Young BH, Peng H, Huard J. Muscle-based gene therapy and tissue engineering to improve bone healing. Clin Orthop 2002(403 Suppl):S243-251.
28. Torrente Y, Camirand G, Pisati F, Belicchi M, Rossi B, Colombo F, El Fahime M, Caron NJ, Issekutz AC, Constantin G, Tremblay JP, Bresolin N. Identification of a putative pathway for the muscle homing of stem cells in a muscular dystrophy model. J Cell Biol 2003;162(3):511-520.
29. Sampaolesi M, Torrente Y, Innocenzi A, Tonlorenzi R, D'Antona G, Pellegrino MA, Barresi R, Bresolin N, De Angelis MG, Campbell KP, Bottinelli R, Cossu G. Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science 2003;301(5632):487-492.
30. Skuk D, Tremblay JP. Cell therapies for inherited myopathies. Curr Opin Rheumatol 2003;15(6):723-729.
31. Skuk D, Tremblay JP. Myoblast transplantation: the current status of a potential therapeutic tool for myopathies. J Muscle Res Cell Motil 2003;24(4-6):285-300.
32. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998;279(5356):1528-1530.
33. Bittner RE, Schofer C, Weipoltshammer K, Ivanova S, Streubel B, Hauser E, Freilinger M, Hoger H, Elbe-Burger A, Wachtler F. Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Anat Embryol (Berl) 1999;199(5):391-396.
34. Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 1999;401(6751):390-394.
35. Gussoni E, Bennett RR, Muskiewicz KR, Meyerrose T, Nolta JA, Gilgoff I, Stein J, Chan YM, Lidov HG, Bonnemann CG, Von Moers A, Morris GE, Den Dunnen JT, Chamberlain JS, Kunkel LM, Weinberg K. Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. J Clin Invest 2002;110(6):807-814.
36. Chen JC, Goldhamer DJ. Skeletal muscle stem cells. Reprod Biol Endocrinol 2003;1(1):101.
37. Bulte JW, Kraitchman DL. Monitoring cell therapy using iron oxide MR contrast agents. Curr Pharm Biotechnol 2004;5(6):567-584.
38. Cahill KS, Gaidosh G, Huard J, Silver X, Byrne BJ, Walter GA. Noninvasive monitoring and tracking of muscle stem cell transplants. Transplantation 2004;78(11):1626-1633.
39. Cahill KS, Germain S, Byrne BJ, Walter GA. Non-invasive analysis of myoblast transplants in rodent cardiac muscle. *International Journal of Cardiovascular Imaging* 2004;20(6):593-598.
40. Walter GA, Cahill KS, Huard J, Feng H, Douglas T, Sweeney HL, Bulte JW. Noninvasive monitoring of stem cell transfer for muscle disorders. Magn Reson Med 2004;51(2):273-277.
41. Hawke TJ, Garry DJ. Myogenic satellite cells: physiology to molecular biology. J Appl Physiol 2001;91(2):534-551.
42. Frimel TN, Walter GA, Gibbs JD, Gaidosh G, Vandenborne K. Noninvasive monitoring of muscle damage during reloading following limb disuse. Muscle Nerve 2005:(In Press).
43. Walter G, Cahill K, Feng H, Douglas T, Huard J, Sweeney HL, Bulte J. Noninvasive Monitoring of Myoblast Transfer for the Treatment of Muscular Dystrophies. Magn Reson Imaging;In press.
44. Bulte JW, Douglas T, Witwer B, Zhang SC, Strable E, Lewis BK, Zywicke H, Miller B, van Gelderen P, Moskowitz BM, Duncan ID, Frank JA. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. Nat Biotechnol 2001;19(12):1141-1147.
45. Bulte JW, Kraitchman DL, Mackay AM, Pittenger MF. Chondrogenic differentiation of mesenchymal stem cells is inhibited after magnetic labeling with ferumoxides. Blood 2004;104(10):3410-3412; author reply 3412-3413.
46. Kostura L, Kraitchman DL, Mackay AM, Pittenger MF, Bulte JW. Feridex labeling of mesenchymal stem cells inhibits chondrogenesis but not adipogenesis or osteogenesis. NMR Biomed 2004;17(7):513-517.
47. Rudin M, Weissleder R. Molecular imaging in drug discovery and development. Nat Rev Drug Discov 2003;2(2):123-131.
48. Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, Basilion JP. In vivo magnetic resonance imaging of transgene expression. Nat Med 2000;6(3):351-355.
49. Wu JC, Chen IY, Sundaresan G, Min JJ, De A, Qiao JH, Fishbein MC, Gambhir SS. Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. Circulation 2003;108(11):1302-1305.
50. Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Genes Dev 2003;17(5):545-580.
51. Min JJ, Gambhir SS. Gene therapy progress and prospects. Gene Ther 2004;11(2):115-125.

52. Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. In vivo visualization of gene expression using magnetic resonance imaging. Nat Biotechnol 2000;18(3):321-325.
53. Moore A, Bonner-Weir S, Weissleder R. Noninvasive in vivo measurement of beta-cell mass in mouse model of diabetes. Diabetes 2001;50(10):2231-2236.
54. Winter PM, Caruthers SD, Yu X, Song SK, Chen J, Miller B, Bulte JW, Robertson JD, Gaffney PJ, Wickline SA, Lanza GM. Improved molecular imaging contrast agent for detection of human thrombus. Magn Reson Med 2003;50(2):411-416.
55. Stegman LD, Rehemtulla A, Beattie B, Kievit E, Lawrence TS, Blasberg RG, Tjuvajev JG, Ross BD. Noninvasive quantitation of cytosine deaminase transgene expression in human tumor xenografts with in vivo magnetic resonance spectroscopy. Proc Natl Acad Sci U S A 1999;96(17):9821-9826.
56. Koretsky AP, Brosnan MJ, Chen LH, Chen JD, Van Dyke T. NMR detection of creatine kinase expressed in liver of transgenic mice: determination of free ADP levels. Proc Natl Acad Sci U S A 1990;87(8):3112-3116.
57. Auricchio A, Zhou R, Wilson JM, Glickson JD. In vivo detection of gene expression in liver by 31P nuclear magnetic resonance spectroscopy employing creatine kinase as a marker gene. Proc Natl Acad Sci U S A 2001;98(9):5205-5210.
58. Gilad AA, McMahon MT, Winnard Jr. PT, Raman V, Bulte JWM, van Zijl PCM. MRI reporter gene providing contrast based on chemical exchange saturation transfer (CEST). Proc Int Soc Magn Reson Med 2005;13:363.
59. Izbudak I, LeRoux MA, Ma LD, Boston RC, Fritzges D, Barry FP, Kraitchman DL. MRI follow-up of knee joint after intra-articular injections of allogeneic mesenchymal stem cells in a caprine model of partial medial meniscectomy. Proc Int Soc Magn Reson Med 2004;12:826.
60. Keinan-Adamsky K, Shinar H, Pelled G, Zilberman Y, Gazit D, Navon G. 1H single and double quantum MRI evaluation of tendon regeneration mediated by engineered stem cells. Proc Int Soc Magn Reson Med 2004;12:2342.
61. Karmarkar PV, Kraitchman DL, Izbudak I, Hofmann LV, Amado LC, Fritzges D, Young R, Pittenger M, Bulte JW, Atalar E. MR-trackable intramyocardial injection catheter. Magn Reson Med 2004;51(6):1163-1172.
62. de Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, Oyen WJ, Bonenkamp JJ, Boezeman JB, Adema GJ, Bulte JW, Scheenen TW, Punt CJ, Heerschap A, Figdor CG. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. Nat Biotechnol 2005;23(11):1407-1413.